X-RAY STUDY OF KERATIN AND OTHER PROTEIN FIBERS[1]

By George C. Nutting[2]

Textile fibers are distinguished from other materials by their high strength and flexibility. Among organic fibers these properties are due to a particular internal structure, a structure in which the molecules are themselves threadlike or fibrous and are arranged much like the fibers in a yarn, that is, largely parallel and almost fully extended.

Because of this structure, the molecules, which are of finite length (like staple fibers) have large areas of sidewise contact. This enables a bundle of molecules to withstand a considerable pull without failure due to slippage of the molecules past one another. Such parallel lining up or "orientation" of fibrous molecules gives the fiber an appreciable part of the tensile strength of the molecules, which individually are so strong as to be almost unbreakable.

Besides orientation, fibers generally exhibit a higher degree of orderliness in the packing together of their molecules. This is often called crystallinity. Crystalline regions or crystallites are small volumes of the fiber in which there is rather perfect fitting together of short sections of a group of molecules. In the crystallites there is a regular periodic arrangement of the atoms that constitute the molecules, making possible the diffraction of X-rays and formation of the X-ray fiber pattern. From the fiber pattern, recorded on a photographic film, it is possible to deduce at least roughly the dimensions of the crystallites, the proportion of crystalline and noncrystalline or amorphous material, the extent of orientation, and the spatial arrangement of the molecular segments inside the crystallites.

These structural data can be related qualitatively to such over-all fiber properties as the breaking elongation, flexibility, boil stability, and tenacity. They help to explain in terms of internal structure why a fiber is good or bad and to suggest the structural change that will modify a fiber's properties in a desired way.

Intrepretation of the X-ray diffraction effects in protein fibers is due largely to Astbury and his colleagues at the University of Leeds (1).[3]

Industrially, keratin, fibroin, and collagen are the most important natural fibrous proteins. All are of animal origin. They are of three structural types, reflecting pronounced differences in the chemical composition of the proteins. The keratins, which include wool, hair, horn, and feathers, are made up of cells, so that caution must be used in correlating whole keratin fiber properties with the structure of the molecular crystallites inside the cells. Fibroin is the protein of silk, the only natural continuous filament fiber. Collagen comes to us not as a fiber at all, but, in hides and skins, as a natural fabric, which is enormously useful after tanning into leather. Collagen also occurs as a thread or even a sort of proteinaceous rope in connective

[1] Adapted from a paper entitled "X-Ray Study of Wool and other Protein Fibers," Presented before Committee D-13 in New York City, March 17, 1949.

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[3] The boldface numbers in parentheses refer to the list of references appended to this paper, see p. 531.

tissue and tendons; and, of course, collagen is the substance composing catgut surgical sutures.

There is a far larger number of globular proteins, all of which are apparently potential fiber formers. They again are threadlike molecules, which ordinarily coil up into globular form. In principle they can be, and in several cases have in fact been, pulled out into an artificially fibrous form (2, 3, 4).

Several globular proteins are available in almost unlimited quantities at a relatively low price. Representative globular proteins are: Casein and lactoglobulin, proteins of milk; egg albumin, a protein of egg white, of interest mainly because

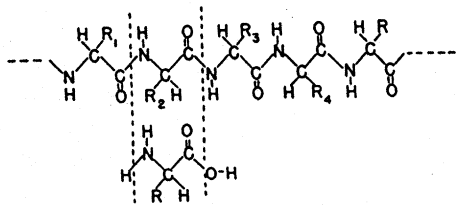

FIG. 1.—Schematic Representation of Part of a Protein Molecule. The Dotted Lines Enclose a Repeating Unit, an "Amino Acid Residue," Derived from an Amino Acid (below) by Removal of Water.

of the ease and completeness with which it can be transformed from the globular into the fibrous form; and zein (the protein of corn), soybean protein and peanut protein, all abundant seed proteins. Zein textile fiber and casein bristle fiber are now being made commercially in this country.

All proteins, globular and naturally fibrous, are polymers of amino acids. Figure 1 is a diagram representing a minute part of a protein molecule. Each H stands for a hydrogen atom, C a carbon atom, O oxygen, and N nitrogen. The lines between the symbols for the atoms represent chemical bonds—pairs of electrons that hold the atoms together. Enclosed in dotted lines is the repeating unit, the "amino acid residue," derived from an amino acid molecule by elimination of the H and OH groups as water. The R's are the "side chain" groups. They may be only hydrogen atoms, or they may be big, bulky groups consisting of a dozen or more atoms. The differences between the R groups are the differences between the amino acids. A single protein may contain as many as twenty amino acids. Proteins are, then, vastly more complex polymers than cellulose and the synthetic fiber formers, which are made up of only one or two chemically different repeating units.

It is deduced from X-ray diffraction that if the "backbone" of the protein molecule—the —C—C—N—C—C—N— repeating zigzag— lies in the plane of the paper the R groups project alternately above and below this plane. If segments of the protein molecules pack sidewise in orderly fashion into a crystallite, it follows that the distance between the backbones of the molecules should be relatively small and constant; whereas, on account of the bulk and the diversity of the R groups, the side chain separation of the molecules, which will be at right angles to the backbone spacing, should be larger and variable.

Figure 2 shows models of a part of molecules of silk and wool. In silk the side chain groups are small. One would expect the X-ray pattern to indicate good crystallinity, and side chain and backbone separation between adjacent molecules to be about equal. For wool, with bulky side groups, one would expect a poorer X-ray fiber pattern and quite different backbone and side chain spacings. The two large atoms in the side chain at the right in the wool model represent sulfur in the amino acid cystine. Cystine is present in unusually large amounts in wool, and affects the fiber properties greatly by forming strong chemical bonds between adjacent wool molecules.

The models pictured contain only seven or eight amino acid residues. If complete, the model for even a relatively small protein molecule like silk would be about 60 times as long as it is in the photograph, whereas the diameter would be the same. The molecule is decidedly threadlike. It is correspondingly flexible, and can be extended until it is almost straight, or can be coiled up into a sphere. If the globular protein molecules can be made fibrous, developing an X-ray fiber

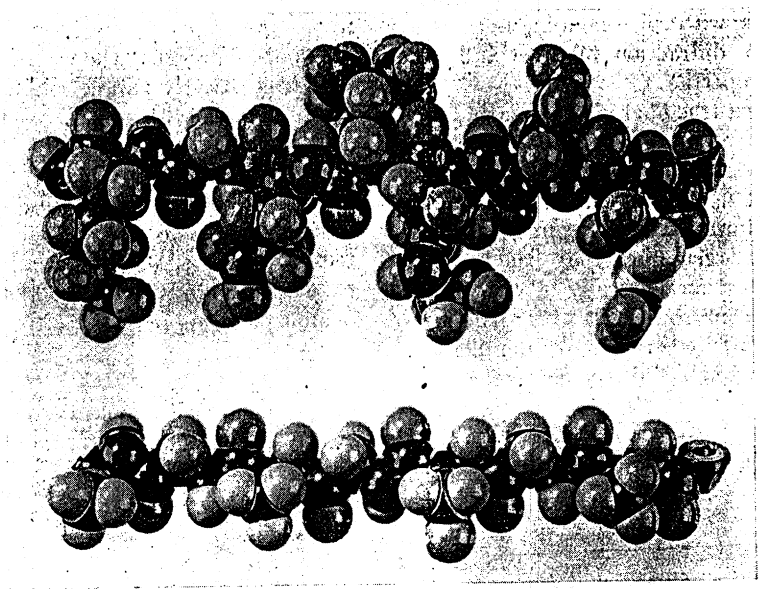

FIG. 2.—Models of Part of a Molecule of Wool (above) and Silk.

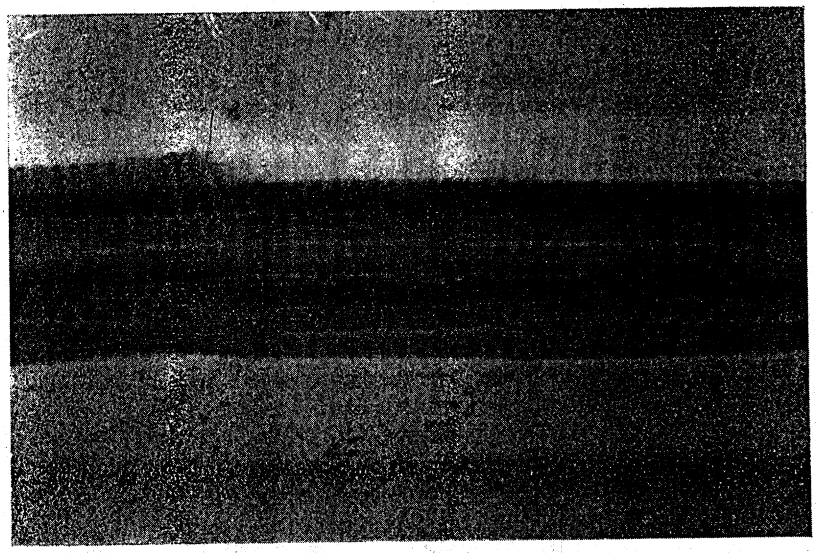

FIG. 3.—Electron Micrograph of Collagen Fibrils. Magnification, 30,000 Diameters.

pattern and improved mechanical properties, and if our ideas of protein structure are correct, we should be able to disorder and disorient naturally fibrous proteins, with corresponding impairment of their diffraction pictures and other fiber properties.

Since a true fiber is molecularly fibrous and oriented, it ought to be cleavable lengthwise into thinner and thinner fiber bundles up to the limit of resolution of the best microscopes. Figure 3 is a picture of a collagen fiber that has been disintegrated mechanically in water into a group of narrow fibrils. The diameter of each fibril corresponds to a parallel array of only about 120 collagen molecules, if

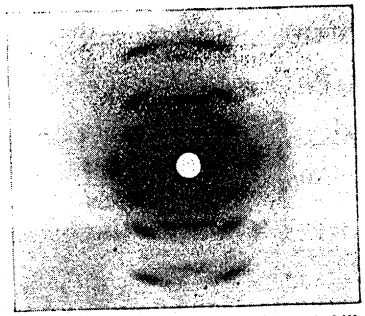

FIG. 4.—X-ray Fiber Pattern of Silk.

measured in the direction of the side chain separation. The cross striations are direct visual evidence of a regular spatial order or crystallinity at almost molecular dimensions.

Figure 4 is the X-ray diffraction pattern of silk, which gives the best diffraction pattern of any protein fiber. The fiber axis is vertical. Spots on the equator correspond to distances between molecules. The strongest equatorial spots are actually double, owing to almost equal separation of molecules in the backbone and side chain directions. The position of spots on the vertical axis, the meridian, indicates the repeating distances along the fiber axis. From the meridional spots, we conclude that the molecular chain c silk is almost fully extended and that th silkworm, when it made the fiber, gave just about the maximum possible stretch to the molecules. The angular length of the spots shows that the greatest departure from strictly parallel orientation of the molecules with respect to the fiber axis is only a few degrees. The large number of spots and their sharpness show that the degree of order within the crystallites is high for a fiber, and the intensity of the spots compared with the hazy background shows that the amount of amorphous material is relatively small.

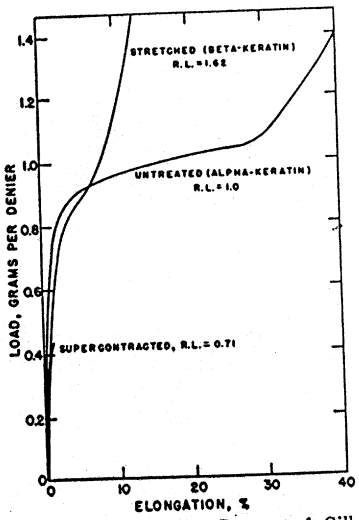

FIG. 5.—Load-elongation Curves of Silk and Collagen.
R. L. = Relative Length

Figure 5 (5) shows load-elongation curves for silk and collagen. The uppermost curve is for the ordinary crystalline and oriented silk whose diffraction pattern is reproduced in Fig. 4. This is a strong fiber, with a tenacity of about 4 g. per denier and with a high elastic modulus. On soaking for a few seconds in formic acid, the silk fiber contracted to 59 per cent of its original length (R. L., "relative length" is 0.59). After being

washed and equilibrated at standard temperature and humidity, it gave the lowest curve. The strength was then only 1 g. per denier, and the elongation was about three times that of the original silk. The X-ray pattern showed a few long, diffuse arcs, indicating great loss in crystallinity and orientation.

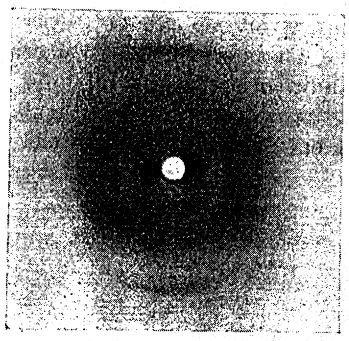

FIG. 6.—Diffraction Pattern of Oriented Collagen.

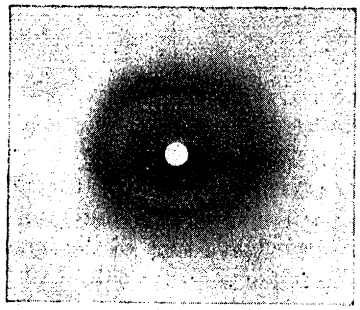

FIG. 7.—Diffraction Pattern of the Alpha-Keratin Structure, for Example, Ordinary Wool or Hair.

The middle curves are a similar pair of load-elongation curves given by native, oriented collagen and the same material contracted and disoriented in warm water. Figure 6 is a diffraction pattern of oriei ted collagen. It shows characteristic distances parallel and perpendicular to the fiber axis. The length of the arcs indicates hightly imperfect orientation, and the diffuseness of the arcs or spots indicates that the crystallites are small and that there is imperfect order within them.

Figure 7 is the X-ray pattern of wool in the normal alpha-keratin form. Compared to silk, this pattern expresses a low order of crystallinity in the fibers, both in perfection and amount. The proportion of crystallites is doubtless low for the fiber as a whole. However, recent electron microscopic observations seem to show that the cortical cells of wool are composite, consisting of a predominant amount of amorphous matrix in which are imbedded oriented and crystalline fibrils (6). The matrix contributes

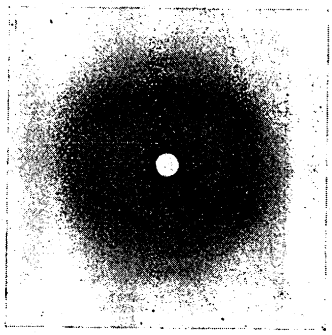

FIG. 8.—Diffraction Pattern of the Beta-Keratin Structure, for Example, Stretched Wool or Egg Albumin.

only the diffuse background to the X-ray pattern, and on this are superimposed the diffraction spots arising from the fibrils. An important characteristic of the pattern is the arc on the meridian, which occurs at a distance that could not correspond to full extension of keratin molecules and is usually explained as denoting regular loops or folds in the molecules. This proposal is attractive because it provides a mechanism for the well-known long-range reversible elasticity of wool.

On stretching wool 50 to 100 per cent, the beta-keratin structure is produced (Fig. 8). The strong equatorial arcs correspond to characteristic backbone and side chain separations, and the position

of the faint meridional arcs indicates that the molecules are completely extended. The beta-keratin structure is the only one in the dozen or so globular proteins that have been made fibrous artificially. Figure 8 is actually the diffraction pattern of egg albumin fiber. The position of the spots is practically identical in the X-ray patterns of all the artificial fibers and all the stretched mammalian keratins. The beta-keratin structure is thus a structural type, within which the amino acid composition of the proteins can

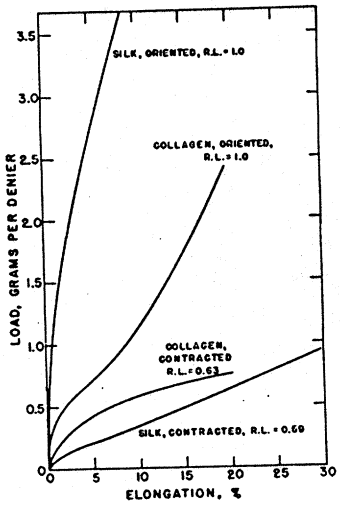

FIG. 9.—Load-Elongation Curves of Keratin in the Form of Hair.

change within wide limits. The structures of silk, collagen, and alpha-keratin have not been reported for any artificial protein fiber.

Figure 9 shows stress-elongation curves for keratin in its three structural forms. Horsehair rather than wool was taken as experimental material simply because of greater ease in handling. The middle curve is for ordinary hair in the alpha-keratin structure. The high elongation is characteristic. The upper curve is for hair stretched 60 per cent and steam-set in the beta-keratin form. The tenacity has been increased about 20 per cent, the elongation has been much reduced, and the fiber has become decidedly brittle. The lower curve is for hair contracted to about 70 per cent of its original length. The orientation and most of the crystallinity have been removed. The X-ray fiber pattern has been lost and the fiber is weak and brittle and has a breaking elongation of only about 2 per cent. Curves for unoriented, partly oriented, and fully oriented egg albumin fibers closely resemble the ones in Fig. 9.

Present-day artificial protein fibers made by a wet spinning process give an

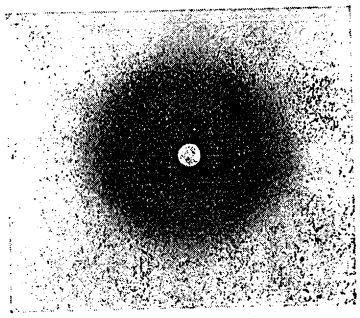

FIG. 10.—Diffraction Pattern of Casein Fiber.

X-ray pattern much like that in Fig. 10. This pattern has two diffuse full rings with very slight arcing of the inner one that corresponds to the side chain spacing in the beta-keratin structure. Crystallinity in these fibers is obviously of a low degree. It cannot be concluded, however, that the protein molecules are unoriented or that they retain their original globular form. By several indirect tests, the molecules are oriented. These tests include the change of tensile properties with stretch in manufacture, the optical double refraction of the fibers, the relative extent of swelling in solvents along and across the fiber, and the thermoelasticity (7). Several other substances composed of threadlike molecules, notably poly-

styrene and polymethyl methacrylate, when formed into oriented fibers also give X-ray patterns composed only of diffuse full rings. This simply illustrates the requirement that there be a fair degree of internal order or crystallinity if X-ray diffraction is used as a measure of molecule orientation.

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